

## Short Communications

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## Isolation of *Staphylococcus aureus* from Raw Cattle Milk and their Drug Resistance Pattern

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### ABSTRACT

*Staphylococcus aureus* is one of the important human pathogens involved in food related diseases and common community associated infections. This organism proliferates in food and causes food-borne illnesses. Milk serves as an ideal medium for growth of many microorganisms including *Staphylococcus aureus*. A total of 118 raw cattle milk samples were collected under aseptic precautions from different places of Navsari district of south Gujarat, processed under standard bacteriological techniques. The Baird Parker Agar was used as selective medium for isolation. The presumptive isolates were identified on the basis of their morphological, cultural and biochemical characteristics. The sensitivity pattern of *S. aureus* with different antimicrobial agents was evaluated by disk diffusion method. Analysis of result revealed 12 isolates (10.16 %) of *S. aureus* from 118 milk samples. The *S. aureus* isolates showed cent percent sensitivity towards Amikacin and Gentamicin, followed in reducing levels by Gatifloxacin (91.66 %), Ciprofloxacin (91.66 %), 75 % each, of Streptomycin and Kanamycin; Ampicillin (66.66 %) and Cephalixin (41.66 %). The pattern clearly indicated overall high percent resistance to Cephalixin (58.33%), followed by Ampicillin (33.33 %), Methicillin (25.00%), Kanamycin (16.66 %) and Gatifloxacin (8.33 %), Ciprofloxacin (8.33 %). The isolates showed intermediate sensitivity towards Streptomycin (25.00 %) and Kanamycin (8.33 %). Findings of the study suggested judicious use of antimicrobial therapy in milking animals.

#### Keywords

Antibiotics, Food, Raw milk, *S. aureus*, Food poisoning

#### Article Info

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### Introduction

Milk is a major component in the human diet of vegetarian class, but it also serves as very good medium for the growth of many microorganisms including pathogenic bacteria (Ruegg, 2003). Milk is a highly valuable food, but raw milk contains and favors growth of many microorganisms. Milk and its

derivates are considered as a major source of *Staphylococcus aureus* infection in man (Jahan *et al.*, 2015). Milk can act as a vehicle for transmitting the *S. aureus* from animal to cause the severe foodborne intoxication in human. It has been recorded that *S. aureus* causes the subclinical mastitis and contaminate the udder and milk; acting as the main source of contaminants. Contaminated

milking equipment's and the milker's hands also may be the source of infection (Capurro *et al.*, 1999).

*Staphylococcus aureus* grow as smooth, circular, convex colonies of 0.5-1.5 µm in diameter with lustrous growth. The colony pigmentation may vary from grey, grey-white, grey- white with yellowish to orange shades and in blood agar typical β-hemolysis may be produced; depending on the growth condition (Jahan *et al.*, 2015).

Microscopic morphology reveals irregular bunches of grapes like clusters of cells. *S. aureus* is Gram +ve, aerobic and facultative anaerobic, oxidase-negative, catalase-positive, non-motile, fermentative, and non-spore forming cocci (Carter *et al.*, 1994). *S. aureus* may be pathogenic or non-pathogenic and the pathogenic strains are usually coagulase-positive and cause disease in their hosts (Jahan *et al.*, 2015). *Staphylococcus aureus* is a versatile pathogen of humans and animals causes a wide variety of diseases from mild skin infection to more severe diseases such as pneumonia and septicaemia (Lowy, 1998).

Antimicrobial resistance is a main public health worry worldwide. The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals (McNamara, 2000). Several authors observed that administration of antibiotics to food producing animals for remedial purposes or as growth promoters may be a primary factor in selecting for antimicrobial-resistant bacteria (Barber *et al.*, 2003).

Thus, the objective of this study was to isolate *Staphylococcus aureus* from raw cattle milk and evaluation of the drug resistance pattern of *S. aureus* isolates using different antimicrobial agents.

## Materials and Methods

### Sample collection

A total of 118 raw milk samples (approximately 10 mL from each) of apparently healthy cow were collected from different places of Navsari district of south Gujarat by using sterile test tube. The samples were collected from March 2016 to June 2016 and investigation was carried out following collection. The laboratory works were accomplished in the laboratories of the Department of Veterinary Public Health And Epidemiology, Navsari Agricultural University, Navsari.

### Isolation and identification of *S. aureus*

The selective medium used for isolation of *S. aureus* was Baird Parker Agar (BPA) (HiMedia). A 0.1 ml of each 10 fold diluted sample was spread on BP agar using a sterile pipette and sterile spreader and incubated for 24-48 hours at 37 °C. The organisms were isolated and identified as methods described by Cowan and Steel (1975), Singh and Prakash (2008). and Quinn *et al.*, (1994). Characteristic appearance of jet black colonies surrounded by a halo zone were considered to be presumptive *S. aureus*. The pure cultures were streaked on Nutrient agar or Mannitol salt agar and incubated for 24 hours at 37 °C and were further characterized by biochemical tests.

### Morphological characteristics

The smear was prepared from the isolated culture on clean grease free microscopic glass slide and stained with Gram's method of staining. The stained smear was observed under microscope. Smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes.

### **Biochemical examination**

Biochemical tests were performed to confirm *S. aureus* using Catalase test, Coagulase test, motility test, DNase test, haemolysin test and D-mannitol fermentation and maltose fermentation.

### **Antibiotic susceptibility assay**

All the *S. aureus* isolates were subjected to antibiotic sensitivity testing by standard disc diffusion method on Muller-Hinton agar (Himedia) according to the National Committee of Clinical Laboratory Standards (NCCLS) recommendations. Drug resistance pattern of the isolates to Amikacin (10µg), Ampicillin (25µg), Cephalexin (30 µg), Ciprofloxacin (30 µg), Gatifloxacin (5µg), Gentamicin (50µg), Kanamycin (30 µg), Methicillin (5µg), Streptomycin (10µg) were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, intermediately susceptible and resistant according to the National Committee of Clinical Laboratory Standards (NCCLS) guideline; Performance Standards for Antimicrobial Susceptibility Testing (NCCLS, 2014).

### **Results and Discussion**

Out of 118 raw milk samples processed, 12 (10.16 %) samples yielded the growth of *S. aureus*, identified based on cultural and biochemical characteristics (viz., Gram-stained smears of the pure cultures exhibited clusters of Gram-positive cocci, positive for catalase and coagulase test and results at par with other biochemical tests). Result of antibiotic sensitivity test revealed that all the isolates were sensitive to Amikacin and Gentamicin, followed in reducing levels of sensitivity by Gatifloxacin (91.66 %), Ciprofloxacin (91.66 %), 75 per cent each of

Streptomycin, Kanamycin and Methicillin; Ampicillin (66.66 %) and Cephalexin (41.66 %). The pattern indicated varying levels of resistance to Cephalexin (58.33%), Ampicillin (33.33 %), Methicillin (25.00%), Kanamycin (16.66 %) and 8.33 per cent each of Gatifloxacin and Ciprofloxacin. The isolates showed intermediate sensitivity towards Streptomycin (25.00 %) and Kanamycin (8.33 %).

In this study we describe the isolation, identification and antibiotic susceptibility characterization of *S. aureus* from raw cattle milk obtained from different places of Navsari district of south Gujarat. The results of present prevalence study in Navsari district of south Gujarat region of India and other author's results from various parts of the world are differing vastly. Even from different regions of India also the prevalence rates were differing. However, results of study results at Navsari district region showed prevalence of 10.16 per cent, which were high compared to other studies conducted by Thaker *et al.*, (2013), who reported 6.25% in Anand (Gujarat) region of India and lower than Kumar and Prasad (2010). Reported 26% prevalence in milk samples collected from local vendors of Pantnagar, India. Sarkar, *et al.*, (2014) documented 74.5% (149/200) of the milk samples were positive for *S. aureus* from the Karnal, North India, and Lingathurai and Vellathurai (2011) reported 61.7% of prevalence of *S. aureus* from 60 raw milk samples from Madurai region of South India; it is very higher than our study.

Results obtained from this study were more or less when compared with the prevalence rates that reported by various authors in different tropical and temperate countries. Prevalence rate from morocco, Palestine and Bangladesh reported by Bendahon *et al.*, (2008), Farhan and Salk (2007) and Jahan *et al.*, (2015) as 40%, 36.9% and 25.53 % respectively, which

were higher to our study. However, similar prevalence has been previously reported by Fagundes *et al.*, (2010) (10.8%) from Sao Paulo state, Brazil, Ayano *et al.*, (2013) (13.8%) from Holeta, Ethiopia. From all these study results of above mentioned indicates prevalence of *S. aureus* is varied from place to place and regions to regions around the world and it highlights that hygienic practice of milking and selling influence the prevalence of *S. aureus* in milk.

According to the obtained results of antibiotic sensitivity tests on the *S. aureus* isolates from raw milk was varied from previous study conducted by Thaker *et al.*, (2013). In this study, the isolates were sensitive to Gentamicin (100%), Ciprofloxacin (91.66 %), Streptomycin (75%), Methicillin (75%); Ampicillin (66.66 %) and Cephalexin (41.66 %). whereas in Thaker *et al.*, (2013) study; it was 90(%), 80(%), 60(%), 100(%), 60(%) and 100(%), respectively. In this study varying levels of resistance to Cephalexin (58.33%), Ampicillin (33.33 %), Methicillin (25.00%), and 8.33 per cent of Ciprofloxacin. whereas in Thaker *et al.*, (2013) study; only 40 per cent resistance to ampicillin while zero per cent resistance reported to Cephalexin, Methicillin, and Ciprofloxacin antimicrobial agent. This difference in the results of various studies indicates antibiotic resistant pattern of *S. aureus* changing. By means of different mechanisms, *S. aureus* developing resistant to different antibiotics day by day. Isolation of antibiotic resistant *S. aureus* from milk samples against these drugs poses a major challenge in human medicine because these drugs are commonly used in the treatment of human.

From the current study, it is noted that intermediate sensitivity towards Streptomycin (25.00 %) against *S. aureus*. %) while in Thaker *et al.*, (2013) study; it was 30 per cent, respectively. if the necessary action not taken

against indiscriminate use of antibiotics, prevalence of antibiotic resistant *S. aureus* may increase further, it may lead to serious health hazards to humans.

Findings of the study suggested judicious use of antibiotics/anti-microbial agents for prophylactic as well as other respectively therapeutic purpose could be the reasons for increased highest antimicrobial resistance of *S. aureus*. A relatively high number of strains are also resistant to the antibiotics commonly used in the therapeutic protocols of many human and animal infections.

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